Impact of Ovarian Hyperstimulation on Thyroid Function in Women with and without Thyroid Autoimmunity

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Pregnancy is accompanied by changes in thyroid function, but limited data are available on these changes in the very first weeks of pregnancy. Yet, T₄ plays a major role in implantation and early fetal development. We sought to determine thyroid function during this period and during the first trimester, in pregnancies achieved by assisted reproductive technology. Furthermore, the thyroid hormone profile was compared between euthyroid women with (TAI+) and without (TAI−) thyroid autoimmunity. We prospectively analyzed data from 35 women who received ovarian hyperstimulation (OH) and presented clinical pregnancies. The mean age of the women was 32 ± 5 yr. Thyroid function tests (serum TSH and free T₄ (FT₄)) and thyroid antibody status were determined before OH (baseline values) and every 20 d after ovulation induction during the first trimester of pregnancy. Serum TSH and FT₄ increased significantly at d 20, compared with baseline values (3.3 ± 2.4 vs. 1.8 ± 0.9 mU/liter; \( P < 0.0001 \) and 13.2 ± 1.7 vs. 12.4 ± 1.9 ng/liter; \( P = 0.005 \)). During the first trimester of pregnancy, there was a significant change over time for TSH and FT₄ (\( P < 0.001 \) and \( P = 0.005 \), respectively). Nine women (27%) were TAI+. The TSH curve among these TAI+ women was significantly higher compared with TAI− women (\( P = 0.010 \)). The opposite was observed for the FT₄ curve (\( P = 0.020 \)).

In conclusion, the present study showed a significant increase of serum TSH and FT₄ levels after OH in the very first period of pregnancy compared with pre-OH levels and a significant impact of TAI on the thyroid hormone profile during the first trimester. This provides evidence for an altered thyroid function in euthyroid TAI+ patients. (J Clin Endocrinol Metab 89: 3808–3812, 2004)

THYROID DISORDERS INTERFERE with several aspects of reproduction (1, 2). Euthyroid women with thyroid autoimmunity (TAI+) have an increased risk for miscarriage, and the prevalence of TAI+ is increased in women with female causes of infertility (i.e., endometriosis, tubal disease, and ovulatory dysfunction) (3–5). The pathophysiology underlying the association between TAI and miscarriage remains to be elucidated. Three major possibilities for the observed association can be considered: first an immune dysfunction (although yet to be defined) can be involved. Second a direct action of antithyroglobulin antibodies (Tg-Ab) on the placenta has been described in mice; to date this has not been described in humans (6). Third, a possible decrease of local thyroid hormones in the presence of TAI during pregnancy can play a role in miscarriage (7).

During pregnancy, the thyroid is submitted to stressors and undergoes several adaptations to maintain sufficient output of thyroid hormones for both the mother and fetus. Changes known to affect thyroid function during gestation are the human chorionic gonadotropin (hCG) peak values (8th–10th weeks), the increased estrogen levels inducing a progressive increase in serum thyroxine-binding globulin (TBG) concentrations, followed in turn by a reduction in free T₄ (FT₄) and a compensatory increase in serum TSH. However, both serum TSH and FT₄ remain within normal reference ranges, unless pregnancy is associated with iodine deficiency (8–10). Ovarian hyperstimulation (OH) used in preparation of assisted reproductive technology (ART) has been shown to impair thyroid function (11). Moreover, in euthyroid pregnant women with positive thyroid antibodies, it has been shown that 16% had increased serum TSH at delivery (12).

By measuring TSH and FT₄ before ART and subsequently every 20 d after ovulation induction (OI; or the end of OH, considered as time 0) during the first trimester of pregnancy, our aim was to investigate changes in thyroid function occurring in the very early phases of pregnancy, i.e. before the impact of high hCG levels. A further aim was to assess the TSH and FT₄ changes over time during the first trimester of pregnancy and to explore the potential impact of TAI+ on thyroid function.

Patients and Methods

Overall study design

Women of infertile couples presenting at the Centre of Reproductive Medicine (Brussels, Belgium) were included prospectively after in-
ART treatment

All female patients received controlled ovarian superovulation treatment by a combination of the GnRH agonist (Suprefact nasal spray, Aventis, Strasbourg, France) and hFSH (Menopur; Ferring Pharmaceuticals, Copenhagen, Denmark) or recFSH (Puregon, Organon International Inc., West Orange, NJ; and Gonal-F; Serono, Geneva, Switzerland). When the patient had at least three follicles with a diameter of 17 mm and serum estradiol levels of 1000 ng/liter, administration of both GnRH agonist and FSH was discontinued, and ovulation was induced with 10,000 IU of hCG (Pregnyl; Organon). All patients had a transvaginal ultrasound-guided ovum aspiration approximately 36 h after hCG injection under local anesthesia.

In conventional in vitro fertilization, each oocyte was inseminated within 3–4 h after retrieval by adding 5,000–20,000 motile spermatozoa per oocyte. For intracytoplasmic sperm injection, only mature metaphase II oocytes were injected after denuding their cumulus cells. The intracytoplasmic sperm injection procedure was carried out as described earlier (13). After fertilization, one to three embryos were transferred depending on their morphological quality. The luteal phase was supplemented by vaginal administration of 3 × 600 mg natural micronized progesterone (Utrogestan; Besins, Brussels, Belgium) starting 1 d after oocyte retrieval.

Pregnancy was diagnosed at least 10 d after transfer by rising hCG levels of at least 20 IU/liter on two occasions. Clinical preg-

Serum assay

Serum TSH and FT4 values were measured using a third-generation electrochemiluminescence immunoassay (Roche, Mannheim, Germany). The reference values were 0.27–4.2 mU/liter for TSH and 9.3–18.0 ng/liter (12–23.2 pmol/liter) for FT4, [conversion factor for FT4 (nanograms per liter → picomoles per liter), 1.29]. Thyroid autoimmunity was demon-

TABLE 1. Baseline characteristics and hCG levels at different time periods after OH for all patients and for patients stratified according to their TAI status*  

<table>
<thead>
<tr>
<th>TAI status</th>
<th>All Patients (n = 35)</th>
<th>Positive (n = 9)</th>
<th>Negative (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>32 ± 5</td>
<td>33 ± 5</td>
<td>31 ± 5</td>
<td>0.205</td>
</tr>
<tr>
<td>TPO-Ab (kU/liter)*</td>
<td>364 (132–3,753)</td>
<td>11 (10–77)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>ET (n)²</td>
<td>2.1 ± 0.6</td>
<td>2.1 ± 0.6</td>
<td>2.1 ± 0.6</td>
<td>1.000</td>
</tr>
<tr>
<td>hCG (IU/liter), d 20</td>
<td>188 ± 121</td>
<td>175 ± 121</td>
<td>193 ± 124</td>
<td>0.708</td>
</tr>
<tr>
<td>hCG (IU/liter), d 40</td>
<td>469 ± 424</td>
<td>431 ± 259</td>
<td>482 ± 473</td>
<td>0.390</td>
</tr>
<tr>
<td>hCG (IU/liter), d 60</td>
<td>19,205 ± 15,059</td>
<td>19,138 ± 17,670</td>
<td>19,231 ± 14,429</td>
<td>0.494</td>
</tr>
<tr>
<td>hCG (IU/liter), d 80</td>
<td>88,217 ± 54,960</td>
<td>76,167 ± 34,678</td>
<td>92,471 ± 59,740</td>
<td>0.270</td>
</tr>
<tr>
<td>hCG (IU/liter), d 100</td>
<td>109,412 ± 77,275</td>
<td>90,500 ± 16,583</td>
<td>115,231 ± 87,961</td>
<td>0.270</td>
</tr>
</tbody>
</table>

Data represent mean ± sd, unless otherwise stated.

* Titters expressed as median (range).
² ET, Number of transferred embryos.
cantly 20 d after OI, compared with baseline values (3.3 ± 2.4 vs. 1.8 ± 0.9 mU/liter; P < 0.0001). Similarly, serum FT₄ increased significantly 20 d after OH, compared with baseline values (13.2 ± 1.7 vs. 12.4 ± 1.9 ng/liter; P = 0.005; Fig. 1, left panels). When stratified according to the presence (TAI+ group) or absence (TAI− group) of thyroid autoimmunity, similar trends were observed, although with the numbers available, these trends did not always reach statistical significance (Fig. 1, right panels).

Thyroid function during the first trimester of pregnancy

Figure 2 shows the pattern of changes in serum TSH and FT₄ during the first trimester of pregnancy in the entire study group (Fig. 2, left panels) and after stratification according to the presence (TAI+ group) or absence (TAI− group) of thyroid autoimmunity (Fig. 2, right panels). In the entire study group, there was a statistically significant effect for time on TSH and on FT₄ (one-way repeated-measures ANOVA for TSH and FT₄, P < 0.001 and P = 0.005, respectively). The peak values for TSH and for FT₄ occurred at d 20 and 40, respectively. When stratified according to TAI status, there was a statistically significant effect, both for serum TSH and FT₄. For serum TSH, the curve was significantly higher among TAI+ women (two-way repeated-measures ANOVA for TSH, P = 0.010). Conversely, for serum FT₄, the curve was significantly lower among TAI+ women (two-way repeated-measures ANOVA for FT₄, P = 0.020).

The outcome of induced pregnancies, as of the end of the study period (first trimester), showed a miscarriage rate of 56 and 39%, respectively, among the TAI+ vs. TAI− groups. These percentages were not statistically different between the groups, due to the small number of patients. Thyroid hormone levels were not statistically different between those (last available) in the miscarriage group and the ongoing group. Mean (±sd) serum TSH levels at time point 80 d was 3.3 ± 2.2 mU/liter in the miscarriage group vs. 2.0 ± 1.3 mU/liter in the ongoing pregnancy group (P = 0.116). FT₄ levels were, respectively, 12.5 ± 1.7 and 13.4 ± 1.5 ng/liter (P = 0.223). At the time point 100 d, serum TSH was 2.9 ± 2.1 mU/liter in the miscarriage group and 2.2 ± 1.5 mU/liter in the ongoing pregnancies group (P = 0.422).

FT₄ levels were 11.9 ± 1.3 and 12.5 ± 1.6 ng/liter, respectively, in the miscarriage and ongoing pregnancy groups (P = 0.509).

Discussion

The present prospective study presented a unique opportunity to study the very early changes in thyroid function during the first trimester of pregnancy after ART and especially during the first month, when hCG levels were still too low (<1000 IU/liter) to directly influence thyroid function. We observed a significant increase in serum TSH and FT₄ levels compared with baseline values. Previously, only Muller et al. (11) investigated thyroid function after OH. These authors measured thyroid function tests in the immediate period after OH but did not precisely specify the timing in relation to the OH, nor did they precisely specify the outcome (pregnancy status) of the patients included. Interestingly, they found a significant increase in serum TSH and a decrease in FT₄ levels compared with pre-OH levels. The postulated mechanism whereby OH leads to these changes in thyroid tests is by inducing a rapid increase in the estrogen levels and in turn TBG production and hyperstimulation, with the latter increasing TBG’s half-life. The increase in TBG results in an increase in total T₄ that tends to lower serum FT₄, thereby stimulating serum TSH through the pituitary-thyroid feedback mechanisms (8). In line with those results, we found significantly increased values for TSH levels at 20 d after OH compared with baseline values. By contrast, in the present study, we found significantly increased values for both TSH and FT₄ serum levels at 20 d after OH (compared with baseline values). The increase in serum TSH might be
related to the high estrogen levels resulting from FSH-induced OH, whereas the injection of hCG for the induction of ovulation might have counteracted the lowering in serum FT4 levels by directly stimulating the thyroid gland (14). A central mechanism to explain these changes in the thyroid hormonal profile is unlikely, because evidence for a direct stimulating effect of estrogen and hCG is lacking at the hypothalamo-pituitary level. The changes in both serum TSH and FT4 during the first trimester were comparable with the thyroid function patterns observed in spontaneous pregnancies. After the early peak value in serum TSH, a decrease in TSH and a further slightly delayed peak value in FT4 are subsequently mediated through rising hCG levels.

A further aim of the study was to assess whether the evolution of thyroid function was similar in women with/without TAI. In a previous case-control study of women from infertile couples, we found an impact of TAI+ on thyroid function, and a clear statistically significant correlation was established between TPO-Ab titers and serum TSH levels before OH. In that study, we also showed that women with a female cause of infertility had an increased risk of being TAI+, compared with fertile controls (5). In a subsequent prospective cohort study of infertile women with TAI+ submitted to OH, we found an increased risk for miscarriage during the first trimester, compared with TAI− women (4).

A similar tendency was found in the present study; namely an increased miscarriage rate in the TAI+ compared with the TAI− group. However, the difference was not statistically different because of the small number of patients investigated. The reasons for such an association remain to be elucidated. Although the hypothesis of a generalized deregulation of immunity is plausible, the development of a relative thyroid dysfunction in women with TAI+ during pregnancy is possible (12). The present study confirmed that there was a difference in the dynamics of thyroid function changes between TAI+ and TAI− women, based on the significantly different serum levels for TSH and FT4 collected at several time periods during the first trimester of pregnancy. The increase in serum TSH was more pronounced, whereas the serum FT4 response was attenuated in TAI+ patients. These results point to a diminished thyroid functional reserve during the ART procedure and subsequent early pregnancy in this subset of patients. Thus, both OH and TAI are factors that can attenuate the normal thyroid response needed for maintaining an ongoing pregnancy after OH.

We also should be aware that reference values for thyroid function are normal values during nonpregnant states and that they may be inappropriate for a certain time point of pregnancy. In the present study, we did not find differences in the thyroid hormone levels between the miscarriage and ongoing group;
However, a larger number of patients should be investigated to be conclusive on this issue. To date, one study investigated the impact of thyroid hormones on the outcome of spontaneous pregnancy in TAI+ women. In that small (n = 16) nonplacebo controlled study, only women with a history of recurrent miscarriage were included. Thyroid hormones before and during pregnancy yielded a significantly better outcome than treatment with Igs during gestation (15).

Clearly, prospective randomized studies comparing the outcome of pregnancy in euthyroid TAI+ women treated by T4 or placebo are needed to answer the remaining question of whether the impaired pregnancy in those patients can be reversed. Previous studies associating TAI+ and thyroid dysfunction with infertility, miscarriage, postpartum thyroiditis, depression, and minor thyroid dysfunction with impaired neuro-intellectual outcome in children (2, 5, 16–19), together with the present study, provide strong evidence to propose systematic screening of infertile women for TSH, FT4, and TPO-Ab before an ART procedure. We also recommend surveillance of thyroid function during subsequent pregnancy in the TAI+ women.

In conclusion, the present study showed a significant increase in serum TSH and FT4 levels after OH in the very first period of pregnancy compared with pre-OH levels and a significant impact of TAI on thyroid function during the first trimester, providing evidence for an altered thyroid function in TAI+ patients.

These changes may be markers of the underlying thyroid alterations possibly associated with the increased miscarriage risk.

Acknowledgments

The authors thank W. Meul and D. Coomans for data support and I. DeWannemacker for the secretarial help.

Received January 22, 2004. Accepted April 13, 2004.

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This work was supported by grants of the Willy Gepts foundation AZ-VUB.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

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